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Manufacturing Variables and Hemostatic Function of Cold Stored Platelets: A Systematic review of the Literature

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ABSTRACT:

BACKGROUND- Platelets for transfusion stored at room temperature (RTP) have a short shelf-life and diminished hemostatic function. Cold stored platelets (CSP) have a longer shelf-life and may be more hemostatically active than RTP. However, the manufacturing techniques used to produce CSP are not standardized. To investigate whether the CSP manufacturing process influences functional characteristics of CSP, we systematically reviewed published CSP articles.

METHODS- MEDLINE and EMBASE databases were searched for articles describing the functional or clinical characterization of CSP published after January 2000. Data relating to manufacturing technique and CSP characteristics in shortlisted articles were recorded using a standardized proforma.

RESULTS- From a total of 1521 articles identified using the search terms, 106 articles underwent full text review, and 33 were shortlisted for full analysis. Marked heterogeneity in manufacturing and functional testing of CSP were identified. A greater relative proportion of articles collecting CSP by apheresis reported higher hemostatic activity than RTP compared to whole blood-derived CSP. A greater relative proportion of articles reporting CSP suspended in plasma alone reported higher hemostatic activity than RTP compared to CSP suspended in plasma plus platelet additive solution. There was no consistent trend suggesting that other manufacturing variables, such as time from collection to cold storage, influenced the functional characteristics of CSP.

CONCLUSION- CSP may have superior hemostatic activity than RTP and therefore, may be more effective at controlling bleeding. However, we highlight that manufacturing technique also influences hemostatic activity and may have an important impact on clinical efficacy and safety of CSP.

INTRODUCTION

The incidence of platelet transfusion increased by 25% in the developed world between 2007-2008 and 2014-2015, predominantly because of wider use in hematological malignancies, which currently accounts for approximately two thirds of all platelets used.[1] Supplying platelets is more logistically challenging than other blood components because with almost all current specifications, platelets require storage at 22°C with continuous agitation (room temperature platelets; RTP). This requires transfusion laboratories to provide a platelet agitator and incubator, thereby increasing costs, logistical burden and equipment footprint. Since room temperature storage also increases the risk of bacterial contamination of platelets, bacterial monitoring of RTP was adopted universally in many countries, further increasing healthcare costs. Even with these measures, the maximum shelf-life of standard RTP is between three and five days in most countries, but may be extended to seven days with point-of-release bacterial testing. The function of RTP also declines during storage because of metabolic changes that impair platelet activation responses, known as the "platelet storage lesion", which is only partially reversible after transfusion.[2-4]

The current use of RTP originated more than four decades ago when several landmark studies compared RTP with platelets stored at 4°C (cold stored platelets; CSP).[5-7] In the first of these studies, autologous Chromium-51 labelled CSP stored *ex vivo* for 18 hours transfused to healthy volunteers had a lower incremental yield and a reduced circulating half-life compared with RTP controls.[5] This study further reported the effect of storage of platelets at 22°C for up to six days and suggested that the yield and half-life of the platelets after transfusion deteriorated with

increasing storage interval but with a rapid deterioration in platelets stored between three and five days.[5] The finding that CSP had unfavorable pharmacokinetic characteristics compared with RTP was reproduced in subsequent studies in healthy volunteers and patients with thrombocytopenia.[7-10] In studies in which functional outcomes were assessed, the bleeding time (BT) corrected more after infusion of autologous CSP than with RTP in aspirin-treated volunteers,[6,7] and in thrombocytopenic patients.[7] However, in other functional studies, there were no differences between CSP and RTP.[8,10,11]

During the decade after these studies, many blood transfusion laboratories stocked both RTP and CSP as a dual inventory. However, since the main indication for platelet transfusion at this time was to prevent bleeding in thrombocytopenic patients, the longer half-life RTP products were favored. Since dual inventories of both RTP and CSP increased complexity and cost, transfusion practice in most centers subsequently altered to a single stock of RTP. Interest in CSP has re-emerged because platelet transfusion is increasingly used to treat acute bleeding, particularly following trauma. In these settings where long term prevention of bleeding is seldom required, the increased hemostatic function of CSP may be clinically advantageous whereas the reduced circulating half-life after transfusion is of less importance. The resurgence in studies relating to CSP has been summarized in several recent reviews which highlight that CSP consistently show superior hemostatic activity by enabling more effective primary hemostasis and by supporting stronger fibrin clot formation compared to RTP.[12-15]

Although together these data create a compelling case for further clinical study of CSP, one important criticism of recent studies is that the CSP manufacturing variables such as collection method, storage medium, and the timing of collection and storage differs between studies. Therefore, the potential impact of manufacturing process on the characteristics of CSP, remains an important area of uncertainty. In order to help resolve this, and to help promote standardization of a future CSP product for clinical study, we have reviewed the current literature with particular focus on manufacturing variables. The review is divided into three core areas:

1. Manufacturing variables used to generate CSP products.
2. The effect of manufacturing variables on the hemostatic function of CSP.
3. The efficacy and safety of CSP in clinical studies.

METHODS

Searches were conducted in January 2018 using MEDLINE and EMBASE databases. The search terms used were: cold AND stor* AND platelet*; "cold platelet*"; refrigat* AND platelet*;("4C" OR "4°C" OR "4 C") AND platelet*. In addition, a search using the Medical Subject Headings (MeSH) “cold temperature” and “blood platelets” was performed in both databases, mapped to the phrase “cold platelet*".

After removal of duplicates, article abstracts were analysed if they reported platelets that were stored in cold, 4°C or refrigerated conditions and fulfilled all of the following inclusion criteria: article published in a peer reviewed journal, reported either apheresis-derived platelets (AP) or whole blood-derived (WBD) platelets, human or

non-human studies, and full text available via institutional accesses (University of Bristol / Defence Medical Services Library Service / British library).

Articles were excluded if they met any of the following criteria: background articles or reviews, abstract only, non-English language articles, platelets cold stored for less than 18 hours, platelets stored in the cold as whole blood (WB), articles published prior to January 2000, or no functional assessment of stored platelets

The articles were screened and shortlisted using Rayyan, a web and mobile app for systematic reviews by two individuals who were blinded to the decisions of the other reviewer.[16] Both sets of shortlisted articles were then compared and a final selection for full text review was agreed. CSP experts were then consulted, and the bibliographies of reviews and primary articles were examined to ensure all relevant articles had been included. To enable focus on current manufacturing practice, literature describing the functional assessment of CSP was restricted to publication dates from January 2000 to January 2018. Manufacturing and functional testing variables were extracted from the screened articles and recorded using a prespecified proforma.

RESULTS

A total of 1521 articles were identified (635 in MEDLINE and 886 in EMBASE) from the search terms described, which included 205 duplicates identified using Endnote duplicate software (Figure 1). Of the remaining 1316 articles, manual inspection of the titles and abstracts identified a further 267 duplicates and a further 943 articles that were excluded for not meeting the eligibility criteria. No additional articles were

identified following consultation with experts in the field or bibliographic review. A total of 106 articles subsequently underwent full text review of which 33 shortlisted articles were confirmed to have met the eligibility criteria and underwent full analysis.

Article characteristics

CSP articles identified in the initial shortlist were published at a rate of 12.6 per year between January 1970 and January 2008, thereafter, increasing rapidly to 72 articles per year in 2017 (Figure 2). CSP publications spanned all five continents with North America and Europe most represented. North American articles predominantly reported use of AP whereas European centers predominantly reported WBD platelets.

Study designs

Of the 33 shortlisted articles that met the eligibility criteria,[17-49] CSP were compared to RTP in 28 (85%) and to temperature-cycled platelets (TCP) in two (6%).[37,48] No CSP comparisons were performed in three (9%) of the articles.[17,22,47] A total of 24 (73%) of the shortlisted articles reported analyses of CSP exclusively *ex vivo*, without administration to a recipient.[18-24,26-36,38,40-42,45,46,49] Of the remaining nine (26%) articles that reported analyses of CSP that were administered to a recipient, two articles reported autologous human donor-recipient transfusion,[18,48] five articles reported human to non-human xenotranfusions,[25,37,39,43,44] one article reported a non-human autologous donor-recipient model,[17] and one article reported a service evaluation of a clinical CSP product.[47]

1. Manufacturing variables used to generate CSP products

Platelet preparation

Seventeen of the shortlisted articles (52%) were collected as AP using devices manufactured by Gambro (1 article),[38] Haemonetics (3 articles)[18,35,43] and Terumo (9 articles),[20,36,37,40,42,45-48] with no manufacturers identified in the remaining 4 articles.[33,39,41,44] In the articles that reported use of AP, CSP were collected into acid-citrate-dextrose (7 articles).[20,38,40,42,45,46,48] The remaining 10 AP articles did not specify an anticoagulant.[18,33,35-37,39,41,44,47] CSP were obtained as an "experimental mononuclear cell collection by-product" (EMBP) in one study and were classified as AP in this review (Table 1).[33]

Of the 19 (58%) articles in which the CSP products were WBD, 10 (53%) articles[23-32] were produced by buffy coat (BC) preparations and nine (47%) were produced via platelet rich plasma (PRP) preparations.[17-22,26,36,39] The predominant anticoagulant for WBD platelets was citrate-phosphate-dextrose which was reported in 10 (53%) articles (Table 1).[19,23,24,26,28-32,36] The two articles that reported the use of CSP from animal origin utilized PRP-derived platelets.[18,25] Three articles reported both AP and WBD CSP products.[18,20,39]

Platelet storage medium and bag

The CSP suspension medium was reported in 28 (85%) of the shortlisted articles. Of these, the suspension medium was plasma alone in 19 (68%),[17-25,40-48] plasma plus platelet additive solution (PAS) in 11 (39%),[26-32,38,40,41] and two articles reporting of both media.[40,41] A total of 12 (36%) articles described supplementation of the suspension medium with additives (Table 1).[17-19,21,23-25,27,38,39,49] Storage bag specification details were included in 21 (64%) articles, of which 20 (95%) reported

use of gas permeable bags.[17,18,21,23,24,26-29,33,34,36-38,40,42,43,45,46] The remaining article reported gas impermeable tubes.[20] Of the 20 articles reporting storage bags, there were 17 different types of storage bags produced by ten different manufacturers.

Time interval between platelet donation and cold storage

The time interval (t) between platelet donation and the start of cold storage was specified in 20 (60%) of the shortlisted articles.[17,18,21-23,25-30,33,35,40-42,44,45,47,48] The time interval was reported as being within 6 hours of donation (t<6h) in 10 articles, [17,18,22,23,26,40-42,45,48] between 6 and 24 hours in three articles (6<t<24h),[28,29,35] and more than 24 hours (t>24h) in seven articles (Figure 3).[21,25,27,30,33,44,48]

Storage conditions and monitoring

A total of 32 (97%) shortlisted articles, reported a CSP storage temperature of 4°C and one reported a temperature of 10°C,[39] which was not considered for any grouped analyses of CSP. Agitation of CSP was reported in 28 (85%) articles, no agitation was used in two (6%) articles,[35,38] and the remaining two (6%) articles compared agitated versus non-agitated CSP.[34,42] CSP were monitored for bacterial contamination in seven (21%) articles.[28,29,34,39,44,47,48]

Manufacture of RTP used as a comparator for CSP

In all of the 29 (89%) articles in which RTP were used as a comparator to CSP, the RTP were stored at 22±2 °C with agitation, but collection and storage methods varied.[18-21,23-46,48,49] However, in 28 articles, the collection and storage methods for RTP were the same as the CSP test product. In the remaining article, RTP were

stored using a gas permeable bag whereas CSP were stored in polypropylene tubes.[43]

2. The effect of manufacturing variables on the hemostatic function of CSP.

Hemostatic function of CSP was tested in all 33 shortlisted articles using a wide range of methods, often with multiple methods reported in the same article (Table 2).[17-49] For reviewing purposes, hemostatic function assays were classified into three categories: *basal activation, activation responses, and dynamic hemostatic function*. Tests to measure the *basal activation* of CSP were performed in 19 (58%) articles and included the use of flow cytometry to measure platelet surface activation markers such as P-selectin, phosphatidylserine or activated $\alpha_{IIb}\beta_3$ integrin (PAC1 antibody binding) in the absence of agonist stimulation.[18,20,21,23,25,27,29,31,35-37,39,40,42-44,46,48,49] In 21 (61%) articles, tests were performed to measure the *activation responses* of CSP to stimulating agonists, using light transmission aggregometry (LTA) in 17 (52%) articles,[18,19,21-23,25,26,30,33,35,37-39,41,46,48,49] and electrical impedance aggregometry (EIA) in four (12%) articles.[41,43-4] In six (18%) articles, CSP *activation responses* were also measured using flow cytometry after agonist stimulation to monitor increases in surface activation markers.[19,21,27,33,46,49] Two articles measured CSP *activation responses* by measuring thrombin generation.[36,46] Twelve (36%) articles tested the *dynamic hemostatic function* of CSP by measuring clot formation and clot strength using viscoelastic tests (e.g. rotational thromboelastometry [ROTEM], thromboelastography [TEG], rheometry)[30,35,40-42,45,47] or by measuring CSP adhesion to a solid surface under shear flow conditions.[20-23,41,46,49]

Evaluation of the physical characteristics of CSP were reported in 16 (48%) articles using visual inspection or microscopy to identify features such as swirl, aggregate formation and the ultrastructure of platelets.[17-19,21,23,25,28-30,35,37,39,41,42,46,47] Analysis of biomarkers in CSP supernatant during storage was reported in 13 (39%) articles.[19,24,25,27,28,31,32,38,40,44,46-48] In the articles that described transfusion of CSP into human or animal recipients, one (3%) reported BT in recipients as a clinical marker of efficacy.[17]

Manufacturing techniques and laboratory test results in CSP versus RTP

In view of the heterogeneity of the 33 shortlisted articles, potential relationships between manufacturing variables and functional test results of CSP (stored at 4°C) compared with RTP were considered only against three main manufacturing variables: AP versus WBD platelets (Table 3), suspension in plasma versus plasma plus PAS (Table 4), and storage within 6 hours of collection (t<6h) versus storage after 6 hours (t>6h) of collection (Table 5).

Basal platelet activation

Of the 18 (53%) articles in which basal activation of CSP was compared to RTP, six (32%) articles showed an increase in basal platelet activation in CSP (increased baseline platelet P-Selectin exposure in five; increased platelet phosphatidylserine exposure in four; increased PAC 1 binding in two).[31,35,42-44,48] By contrast, 12 (58%) articles showed no difference in basal platelet activation between CSP and RTP (Table 3).[18,20,21,23,25,27,29,36,37,40,46,49]

Manufacturing variables were not reported in all of these articles. However, a higher relative proportion of the six articles that did report higher basal activation with CSP used AP (5/6, 83%), plasma suspension (4/5, 80%) and t>6h (2/3, 66%). By contrast, a higher relative proportion of the 12 articles that reported no difference between CSP and RTP used WBD (8/12, 67%) platelets, suspended in plasma plus PAS (4/9, 44%) and stored within 6 hours (t<6h) after collection (6/10, 60%; Table 3).

Platelet activation responses

Of the 20 (60%) articles in which activation responses of CSP were compared with RTP, 14 (70%) articles showed increased activation responses in CSP (increased LTA responses in eight;[21,26,30,33,38,41,46,48] increased EIA responses in four;[42,44-46] increased platelet surface activation markers after agonist stimulation in three,[21,33,46] and increased thrombin generation in two).[36,46] One article reported increased activation responses in RTP compared to CSP after platelets were dual-stimulated with both ADP and collagen, but increased activation responses in CSP after stimulation with either ADP or collagen alone.[37] The remaining six (30%) articles showed no difference in activation responses between CSP and RTP (Table 4).[22,23,25,27,35,49]

Manufacturing variables were not reported in all of these articles. However, a higher relative proportion of the 14 articles that reported increased CSP activation responses used AP (10/14, 71%), suspended in plasma (7/10, 70%) and stored within 6 hours (t<6h) after collection (6/10, 60%). By contrast, a higher relative proportion of the six articles that reported no difference between CSP and RTP used

WBD (5/6, 83%) platelets, suspended in plasma (3/5, 60%) and stored more than 6 hours (t>6h) after collection (3/5, 60%; Table 4).

Platelet dynamic hemostatic function

Of the 12 (36%) articles in which dynamic hemostatic function of CSP was compared with RTP, six articles showed increased dynamic hemostatic function in CSP (increased viscoelastic strength in four;[35,40,42,45] increased aggregation under shear conditions in one,[46] and increased clot strength by rheometry in one).[41] The remaining six articles showed no difference in dynamic platelet hemostatic function between CSP and RTP (Table 5).[21,23,30,32,47,49]

Manufacturing variables were not reported in all of these articles. However, a greater relative proportion of the six articles that reported higher dynamic hemostatic function with CSP used AP (6/6, 100%), suspended in plasma (3/5, 60%) and stored within 6 hours (t<6h) of collection (5/6, 83%). By contrast, five of the six articles that reported no difference in dynamic hemostatic function used WBD (83%) platelets.[21,23,30,32,49] Amongst the articles with no difference in dynamic hemostatic function, the relative proportions of articles with suspension in plasma versus plasma plus PAS and those with storage within 6 hours (t<6h) of collection versus storage 6 hours after collection (t>6h) were the same (Table 5).[21,23,30,32,47,49]

Within-study comparisons of CSP manufacturing variables

Of the 33 shortlisted articles, 11 (33%) compared at least two CSP products within the study, that were manufactured in different ways.[17-19,21,25,34,36,40-42,49] Six of these articles reported the effect of additives in the CSP storage medium. One article that

used glucose-free medium with antimycin A to suppress CSP metabolism reported increased CSP basal P-Selectin expression and adhesion to vWF and fibrinogen compared to HEPES-Tyrode's medium.[49] Of the remaining five articles that compared additives, one article reported no difference between Thrombosol and amiloride plus sodium nitroprusside[19]; three articles reported no effect from UDP-Galactose, used to galactosylate the platelets.[18,21,25] In an autologous baboon transfusion model, thrombopoietin or cytochalasin B plus ethylene glycol tetraacetic acid (cyto-EGTA) did not alter the CSP recovery or survival.[17]

In one article, microaggregates were observed in CSP suspended in plasma, but not in plasma with PAS (30:70).[40] In a similar article, CSP in plasma plus PAS (30:70) formed clots with less crosslinking but similar stiffness compared to control CSP in plasma alone.[41] Two articles reported no differences between agitated and non-agitated CSP.[34,42] One article reported that thrombin generation with apheresis-derived CSP peaked earlier than with WBD CSP, while all other thrombin generation profile parameters and functional characteristics remained comparable.[36]

3. The efficacy and safety of CSP in clinical studies

Of the 33 shortlisted articles, nine articles (27%) reported CSP transfusion to human or animal recipients followed by enumeration or functional characterization of circulating platelets obtained from the recipient after transfusion.[17,18,25,37,39,43,44,47,48]

In a single, open-label phase 1 study, four healthy human volunteers received radiolabelled autologous apheresis-derived CSP stored for 48 hours that were either galactosylated with UDP-galactose or untreated.[18] The mean survival of the

galactosylated CSP (2.2 ± 0.9 (SD) days) were the same as non-galactosylated CSP controls (2.9 ± 0.1 days), but both CSP products had shorter survival than RTP (>6 days).[18]

In the second study, healthy volunteers also received radiolabelled autologous platelet transfusions.[48] In this study, double collections of AP suspended in plasma were stored as RTP, CSP, or TCP (TCP – 24 hours at room temperature followed by continuous cycling between 11 hours at 4°C and 1 hour at 37°C) for 7 days. Paired comparisons between RTP and TCP or TCP and CSP were performed through radiolabelling with either ^{51}Chromium or ^{111}Indium before re-infusion and calculation of recovery and survival. CSP were reported to have the poorest recovery with TCP being better than CSP, but TCP not being equivalent to RTP.[48]

One article described the implementation of a CSP programme at a single US centre for trauma patients.[47] The article describes administration of 21 CSP units (all AP, suspended in plasma and stored within 6 hours) to 20 recipients. In this programme, 80.9% of CSP were discarded either because of the presence of visible platelet aggregates or because CSP had reached the end of the 72-hour shelf-life (as stipulated by the 2015 Food and Drug Administration (FDA) license).[47] No functional or outcome data was reported in this article.

Of the six articles reporting CSP transfusion to animal recipients, a single article compared autologous transfusion in baboons with CSP stored with either thrombopoietin, cyto-EGTA, or no additives.[17] The platelet recovery and survival was the same in the treatment groups and the prolonged BT caused by aspirin treatment

corrected similarly in thrombopoietin-treated and no-additive CSP models.[17] Five articles reported human CSP transfusion into immunosuppressed mice or rats.[25,37,39,43,44] In all cases, CSP had reduced recovery and survival compared to RTP. There were no adverse events from CSP in any of the human or animal CSP recipients, including bacterial contamination or sepsis.

DISCUSSION

A striking feature of this review was that amongst the 33 shortlisted articles, there was wide variation in the manufacturing techniques for CSP. There were also marked differences in the way that different CSP products were functionally evaluated. Most of the articles compared CSP from a single manufacturing process to RTP controls rather than comparing CSP prepared from different manufacturing techniques. Few articles reported transfusion of CSP into recipients, and very limited data described the functional characteristics of CSP after transfusion.

Despite these limitations, several broad observations can be drawn from this analysis. First, AP and WBD platelets were both common ways of preparing CSP in the shortlisted articles. Articles reporting AP CSP usually showed higher hemostatic activity than RTP controls whereas articles reporting WBD CSP usually showed no difference to RTP. Second, suspension of CSP in plasma alone was more common than suspension in plasma plus PAS in the shortlisted articles. Articles reporting CSP suspended in plasma alone usually showed higher hemostatic activity than RTP controls. Articles reporting CSP suspended in plasma plus PAS usually showed no difference to RTP but less platelet aggregate formation. Third, cold storage within 6 hours ($t<6h$) and cold storage after 6 hours ($t>6h$) of platelet collection were both

common techniques in the reported articles. There was no consistent trend between time to cold storage and hemostatic activity of CSP versus RTP. Fourth, there was no consistent trend suggesting that other manufacturing variables such as agitation versus non-agitation, additive versus no additive, or storage bag specification influenced the functional characteristics of CSP.

The observation that most articles who used AP products reported higher hemostatic activity in CSP than RTP controls, whereas articles who used WBD platelets saw no difference between CSP and RTP controls has not been demonstrated previously. Differences in platelet activation levels among AP, PRP, and BC-derived platelets have been reported pre-storage; for example, platelet basal activation exposure detected by measuring CD62P, GPIIb/IIIa activation, and GPIb shedding have all been shown to occur less frequently with the collection of AP compared to PRP-derived platelets.[50-52] A proposed explanation is that less mechanical manipulation is required for preparation of AP compared to WBD, potentially leading to lower levels of *ex vivo* platelet activation and depletion of pro-activation mediators such as platelet granules and surface receptors[50,51] However, following AP and WBD platelet storage at room temperature, no discernible differences in clinical hemostatic efficacy, recovery, or safety endpoints have been proven.[50,51] The possible differences between AP and WBD CSP could result from the varying degrees of pre-storage activation being preserved in CSP, whereas in RTP the differences disappear as the platelet storage lesion evolves.

The observation that most articles reporting CSP suspended in plasma alone showed higher hemostatic activity than RTP controls whereas CSP stored in plasma

plus PAS were usually no different than RTP controls is similar to those reported by Van Hout et al*. in vitro*.[53] In this article whole blood was reconstituted using components: packed red blood cells, plasma, and either RTP in plasma alone or RTP suspended in plasma and PAS (30:70). Response to agonists was reduced in WB reconstituted using RTP in plasma plus PAS compared to RTP in plasma alone.[53] It has also been shown in CSP that the addition of PAS to plasma, reduces aggregate formation in the storage bag as well as fibrin crosslinking within the clot *in vitro*.[40,41] This result may be due to reduced binding of FXIIIa and fibrinogen in CSP suspended in plasma plus PAS compared to plasma alone.[40,41] Practically there are pros and cons of both CSP stored in plasma alone versus CSP stored in plasma plus PAS. For example, 100% plasma CSP are more likely to form aggregates, which in clinical practice contributes to high wastage.[47] Conversely, the addition of PAS to plasma not only may result in reduced hemostatic function of CSP, but also increases the crystalloid volume transfused during the resuscitation, potentially resulting in inferior clinical outcomes.[53]

The observation of no consistent trend between time to cold storage and CSP hemostatic activity versus RTP is consistent with the outcome of a recent study that was published after the searches for this review were performed. This reported that refrigeration of platelets after four days storage at room temperature can restore hemostatic function to a comparable level as CSP cooled the day after collection.[54] This article would support the finding that the time from collection to cold storage is the least critical variable of the three analyzed here. It is important to highlight however, because only 60% of articles reported the time from collection to cold storage, any real differences in hemostatic function may have been lost in this

analysis. Indeed, an explanation for the observed trend of higher hemostatic activity seen in apheresis-derived CSP could in part be due to the compound effect of early storage in the cold and collection by apheresis and conversely, delayed cooling with WBD CSP. It would be important to consider if the time to cooling of platelets following collection independently influences CSP functional outcomes when performing further CSP studies and clinical trials.

Limitations of this analysis

The most significant limitation of this review is the heterogeneity of the articles analyzed. There was also incomplete reporting of important manufacturing variables, making the number of articles available for comparison small. Variation in the methods used to assess the function of CSP required grouping of methodologies to enable any conclusions to be drawn; although these groups generalize complex assays their principles are similar. The lack of functional data is most apparent in the clinical studies, in which no studies directly compared RTP and CSP clinical outcomes. The last article comparing clinical outcomes in patients following RTP versus CSP transfusion was published 38 years ago, and there is a real need for clinical trials to understand the impact of CSP in patient populations.[9]

CONCLUSIONS

This review highlights that CSP may have superior hemostatic activity compared to RTP and therefore, may be more effective clinically in some applications, particularly in acute bleeding where sustained survival of transfused platelets in recipients is not essential. However, we also highlight that manufacturing technique influences hemostatic activity and may have an important impact on clinical efficacy and safety

of CSP. Operational considerations are also present which may potentially influence manufacturing techniques. The systems worldwide for provision of blood and its components vary hugely from small scale centres to large scale, nationalized systems. It is therefore unlikely that a single CSP manufacturing methodology will be universally adopted without robust evidence of benefit.

We highlight that there is an unmet need for randomised clinical trials investigating the efficacy of CSP compared to current RTP products in each of the clinical groups in which platelet transfusion is frequent, particularly trauma, cardiothoracic surgery and hemato-oncology patients. It is essential for future RCTs to examine clinically relevant endpoints such as morbidity and mortality, or healthcare cost outcomes rather than laboratory endpoints. Moreover, significant efforts should be made to standardise manufacturing variables across trials and that CSP products should be developed in collaboration with blood bank services to ensure that findings can be readily translated to healthcare services.

FIGURE LEGENDS

Figure 1: Results from the literature searches, screening and full text review.

Figure 2: Number of published articles by year. Data represent the total number of articles identified in the initial search after removal of duplicates (n=1049).

Figure 3: Time taken from donation to the start of cold storage. Data are from 21 articles in which time from donation to storage was reported and are subdivided according to method of manufacture of CSP: AP- Apheresis derived; WBD PRP – Platelet rich plasma; WBD BC-buffy coat.

TABLE TITLES

Table 1: **Platelet suspension medium, anticoagulant and additives**.

Table 2: **Functional and quality tests reported in the shortlisted articles.**

Table 3: **Baseline platelet activation markers in CSP compared to RTP.**

Table 4: Activation response to agonist stimulation in CSP compared to RTP.

Table 5: Dynamic hemostatic function in CSP compared to RTP.

Table 1

	AP	WBD BC	WBD PRP
Total (n=33)	**17**	**10**	**9**
Anticoagulant			
Acid-citrate-dextrose solution A (ACD)	**7**[20,38,40,42,45,46,48]	**0**	**3**[17,20,22]
Citrate-phosphate-dextrose (CPD)	**0**	**8**[23,24,26,28-32]	**2**[19,36]
Citrate	**0**	**0**	**1**[26]
Not reported	**10**[18,33,35-37,39,41,43,44,47]	**2**[25,27]	**3**[18,21,39]
Suspension medium			
Plasma	**10**[18,40-48]	**3**[23-25]	**6**[17-22]
Plasma and PAS	**2** PAS[40,41] **1** PAS III M[39] **1** Tyrodes [38]	**1** PAS[26] **3** T-SOL[27-29] **3** SSP+[30-32]	**0**
Not reported or other medium	**5**[33-37]	**0**	**1**[36]
Additives			
ThromboSol (LifeCell Corp.)	**0**	**3**[23,24,27]	**1***[19]
Trehalose	**2**[38,39]	**0**	**0**
Modifiers of Galactosylation	**1**[18]	**1**[25]	**2**[18,21]
Amiloride and Sodium Nitroprusside	**0**	**0**	**1**[19]
Thrombopoeitin	**0**	**0**	**1**[17]
Glucose free medium + antimycin A	**0**	**0**	**1**[49]
Cytochalasin B and L EGTA-AM	**0**	**0**	**1**[17]

Where >1 platelet origin or manufacture method was used, all methods are described. * ThromboSol without Ticlopidine.

Table 2

TEST	AP	WBD BC	WBD PRP
BASAL PLATELET ACTIVATION			
Platelet surface markers by flow cytometry, unstimulated (n=22)	**11**[18,34-37,39,40,42,44,46,48]	**5**[25,27,29,31,32]	**8**[18-22,36,39,49]
PLATELET ACTIVATION REPONSE			
Light transmission aggregometry (n=17)	**9**[18,33,35,37-39,41,46,48]	**4** [23,25,26,30]	**5**[19,21,22,39,49]
Impedance aggregometry (n=4)	**4**[42,44-46]	**0**	**0**
Activation responses by flow cytometry following agonist stimulation (n=6)	**2** [33,46]	**1**[27]	3[19,21,49]
Thrombin generation test (n=1)	**1**[36]	**0**	**0**
DYNAMIC HEMOSTATIC FUNCTION			
Viscoelastic test (n=6)	**5**[35,40,42,45,47]	**1**[30]	**0**
Adhesion in shear conditions (n=7)	**2**[41,46]	**1**[23]	**4**[20-22,49]
Rheometry (n=1)	**1**[41]		
OTHER			
Physical characteristics (n=15)	**7**[18,37,39,41,42,46,47]	5[23,25,28-30]	**5**[17-19,21,39]
Hypotonic shock response (n=4)	**2**[18,35]	**1** 30	**2** 18,19
Supernatant biomarkers (n=13)	**6**[38,40,44,46-48]	**6**[24,25,27,28,31,32]	**1**[19]
***In vivo* function and/or recovery and survival (n=8)**	**6**[18,37,39,43,44,48]	**1**[25]	**3**[17,18,39]

Methods used to assess CSP function are subdivided according to whether the CSP were apheresis derived (AP), whole blood derived and buffy coat (WBD BC) or whole blood derived and platelet rich plasma WBD PRP).

Table 3

Reference	MANUFACTURING VARIABLE			BASAL PLATELET ACTIVATION		
	AP (Y/N)	Plasma alone (Y/N)	Cooling <6hrs (Y/N)	More with RTP	No difference	More with CSP
[27]	N	N	N		+	
[23]	N	Y	Y		+	
[49]	N	N	-		+	
[29]	N	N	N		+	
[21]	N	Y	N		+	
[18]	Y	Y	Y		+	
[25]	N	Y	N		+	
[35]	Y	-	N			+
[43]	Y	Y	-			+
[42]	Y	Y	Y			+
[44]	Y	Y	N			+
[46]	Y	Y	Y		+	
[31]	N	N	-			+
[36]	Y	-	-		+	
[36]	N	-	-		+	
[37]	Y	-	Y		+	
[40]	Y	Y	Y		+	
[40]	Y	N	Y		+	
[20]	N	Y	-		+	
[48]	Y	Y	-			+

The studies listed in chronological order have been classified according to whether basal activation was more with CSP, no difference between CSP and RTP or more with RTP. The main manufacturing variable have been classified as follows: apheresis platelets (AP) Y versus whole blood derived (N); suspended in plasma alone (Y) versus plasma plus additive solution (N); cooled <6 hours after collection from donor (Y) versus more than 6 hours (N).

Table 4

Reference	MANUFACTURING VARIABLE			PLATELET ACTIVATION REPONSE		
	AP (Y/N)	Plasma alone (Y/N)	Cooling <6hrs (Y/N)	More with RTP	No difference	More with CSP
[27]	N	N	N		+	
[23]	N	Y	Y		+	
[33]	Y	-	N			+
[49]	N	N	-		+	
[21]	N	Y	N			+
[26]	N	Y	Y			+
[25]	N	Y	N		+	
[38]	Y	-	-			+
[35]	Y	-	N		+	
[22]	N	Y	Y		+	
[42]	Y	Y	Y			+
[44]	Y	Y	N			+
[45]	Y	Y	Y			+
[30]	N	N	N			+
[36]	Y	-	-			+
[36]	N	-	-			+
[37]	Y	-	Y	+*		+
[40]	Y	N	Y			+
[46]	Y	Y	Y			+
[41]	Y	N	Y			+
[48]	Y	Y	-			+

Data are presented in the same way as Table 4 but describe the results of tests to measure platelet activation response. *Dual agonist stimulation (ADP and Collagen).

Table 5

Reference	MANUFACTURING VARIABLE			DYNAMIC HEMOSTATIC FUNCTION		
	AP or WBD	Plasma alone (Y/N)	Cooling <6hrs (Y/N)	More with RTP	No difference	More with CSP
[23]	N	Y	Y		+	
[49]	N	N	-		+	
[21]	N	Y	N		+	
[35]	Y	-	N			+
[42]	Y	Y	Y			+
[45]	Y	Y	Y			+
[30]	N	N	N		+	
[46]	Y	Y	Y			+
[40]	Y	N	Y			+
[41]	Y	N	Y			+
[47]	Y	Y	Y		+	
[32]	N	N	-		+	

Data are presented in the same way as table 4 but describe the results of tests to measure platelet dynamic hemostatic function.

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